Neuroprotective and neurodegenerative effects of the chronic expression of TNF in the substantia nigra of adult mice

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Colloquium C06: Gene Transfer to the Nervous System: From Basic Mechanisms to Novel Therapeutics

C06-01

Gene therapy for nervous system repair

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Using experimental models of neurotrauma, several laboratories have demonstrated the efficacy of neurotrophic factors (NFs) to protect neurons from death and to support axonal regeneration. These observations raised the possibility that NFs may be effective in treating neurotrauma and neurodegenerative diseases in humans. However, clinical applications of NFs have been restricted as systemic delivery of NFs at doses sufficient to reach therapeutic concentrations and to overcome their short half-lives cause adverse effects. These limitations would be overcome if NFs were continually expressed at the target site. We tested whether local expression of NFs would induce axonal regeneration in a model of neural injury. The cortical spinal tract (CST) in rats was lesioned unilaterally at the level of the hindbrain and Neurotorphin-3 (NT-3) was expressed locally in the spinal cord either by retrograde delivery of the vector from the sciatic nerve. Axons were observed growing from the unlesioned CST across the midline to the denervated side. Interestingly, axons did not cross the midline if the CST was not lesioned suggesting that the effect of NT-3 was dependent upon trauma-induced signals. These data demonstrate that local expression of NFs will induce and support axonal regeneration in a circumscribed area after injury without adverse effects and suggest that a therapy based upon this strategy may overcome the limits of systemic NF delivery for SCI.

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C06-02

The neuroimmunology of viral vectors for gene transfer: a rational approach to neurological gene therapy

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Vectors, such as adenovirus and lentivirus-derived vectors, are powerful tools to transfer genes into the brain, for either basic science or therapeutic aim. Various types of vectors have now been shown to allow up to 12–18 months of stable transgene expression in the brain. However, injection of viral vectors into the brain can cause brain inflammation. This inflammation is acute, reversible within 30 days, and unable to inhibit transgene expression. Also, transgene expression can be 'eliminated' by the adaptive arm of the immune system, once the immune system is primed through a systemic immunization protocol. The cellular and molecular mechanisms of viral vector induced inflammation, as well as the cellular and molecular mechanisms used by cells of the adaptive immune system to abolish transgene expression in the brain have not yet been completely elucidated. Understanding these phenomena is complicated by the so called 'brain immune privilege'. This presentation will discuss the interactions of viral vectors with both the innate and adaptive immune response, and propose how an understanding of the basis for the brain's immune privilege can aid in the development of gene therapies for brain disorders, both for chronic neurodegeneration, such as Parkinson's disease using growth and differentiation factors, or brain tumors, using immune-stimulatory strategies.

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C06-03

Neuroprotective and neurodegenerative effects of the chronic expression of TNF in the substantia Nigra of adult mice

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Activation of microglia and induction of cytokines such as Tumor necrosis factor alpha (TNF) in the substantia nigra (s.n.) have been observed in animal models and patients with Parkinson's disease (PD). However, it is unclear whether this microglial activation could be functional to remove cellular debris after neuronal death or could be triggering, propagating or delaying the death of dopaminergic neurons. We have generated conditional knock-in mice in which a TNF transgene is under the control of the endogenous engrailed promoter (which directs protein expression mainly to the s.n.). In these mice, constitutive TNF expression is reduced by an interfering cassette that is flanked by loxP sequences. Injection of adenoviral vectors expressing CRE (AdCRE) in the s.n. resulted in removal of the interfering cassette. In addition, s.n.-specific TNF up-regulation was seen in the AdCRE-injected knock-in mice but not in control animals. Using this combination of techniques, we showed that while chronic up-regulated TNF expression induced progressive neuronal loss, lower TNF levels were neuroprotective in the s.n. in the 6-OHDA model of PD. These data suggest a dual role of the chronic expression of TNF in the s.n. In addition, the progressive neurodegenerative effect of TNF *per se* in the s.n. provided us with a new animal model of PD.

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C06-04

Functional consequences P/Q-type calcium channel ablation

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P/Q ($Ca_v2.1$) calcium channels are expressed throughout central and peripheral nervous system in most presynaptic terminals where they play a prominent role in controlling neurotransmitter release. Mutations in the CACNA1A, the gene encoding the pore-forming α1A subunit of P/Q-type calcium channel, cause a group of human neurological disorders characterised by ataxia, migraine and/or epileptiform activity. To understand the consequences of P/Q-type calcium channels deficiency we studied transmitter release in mice whose α1A subunits were completely deleted (Jun *et al*., 1999 *PNAS* **96**, 15,245). As anticipated, P/Q-type calcium channels were eliminated from whole cell recordings of calcium currents in cultured chromaffin cells. Alterations in synaptic transmission like reduction in quantal output, altered synchronisation and lack of short-term facilitation were present at the neuromuscular junction (Urbano *et al*., 2001 *Biophys. J*. **80**, 940). This suggests that α1A–/– mice compensate the deficiency of P/Q-type calcium channels with the over expression of other types of calcium channels. In an attempt to express P/Q-type calcium channels, we infected α1A–/– cromaffin cells '*in vitro*' and α1A–/– cerebellar cells '*in vivo*' with a vector derived from the herpes simplex virus type-1 containing the transgen of α1A-GFP fusion protein. The expression of the protein was confirmed by the measurement of GFP fluorescence.